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TO:	FROM:
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COMPANY:	DATE:
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☒ URGENT ☐ FOR REVIEW ☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE

NOTES/COMMENTS:

Dear Examiner Nguyen:

These attachments will be used in the interview on Friday, October 4, 2002, at 9A.M. There is no need to review them prior to the interview. You may want to look at proposed claims 100(A) and 100(B).

A method for isolating exon I of a gene comprising:

(a) transfecting one or more eukaryotic cells *in vitro* with the vector of any one of claims 58, 61, 65, or 67;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

(c) selecting for cells in which said vector has transcriptionally activated an endogenous gene containing one or more exons;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA;

(f) recovering a cDNA molecule containing vector sequence and exon sequence from said endogenous gene; and

(g) using the exon sequence in the endogenous gene in (f) to obtain a cellular transcript or cDNA of a cellular transcript that contains the endogenous gene exon sequence but not vector sequence.

A method for isolating exon I of a gene comprising:

(a) transfecting one or more eukaryotic cells *in vitro* with the vector of any one of claims 58, 61, 65, or 67;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

(c) selecting for cells in which said vector has transcriptionally activated an endogenous gene containing one or more exons;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA;

(f) recovering a cDNA molecule containing vector sequence and exon sequence from said endogenous gene; and

(g) using the exon sequence in the endogenous gene to obtain genomic DNA containing exon I of the endogenous gene.

Applicant's Copy

DETAILED ACTION

1. Claims 1-7, 10-15, 20-36 and 58-231 are pending in the instant application

Election/Restriction

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-4, 10-12, 15, 20-24, 28-29, 85, 86, 128, 159, 161-162, 164-167, 169-175, 177-183, and 214, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

II. Claims 5-7, 13-14, 20-24, 28-29, 85, 128, 159, 161-162, 164-167, 169-175, 177-183, and 227, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

III. Claims 1-4, 10-12, 15, 20-24, 28-29, 85, 86, 128, 159, 161-162, 164-167, 169-175, 177-183, and 214, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

IV. Claims 5-7, 13-14, 20-24, 28-29, 85, 128, 159, 161-162, 164-167, 169-175, 177-183, and 227, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

V. Claims 30-35, drawn to primers, classified in class 536, subclass 24.33.

VI. Claims 36, drawn to a method of DNA synthesis, classified in class 435, subclass 91.3.

VII. Claims 58, 59, 64-69, 71-74, 76-82, 85-123, 128, 129, 130-132, 157, 159, 161-162, 164-167, 169-175, 177-183, and 223-226, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

VIII. Claims 60, 63-86, 99-115, 128, 130-132, 197, 203, and 223-224, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

IX. Claims 61, 63-86, 99-115, 128, 130-132, and 223-224, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

X. Claims 62-82, 99-115, 128,130-132, 159, 161-162, 164-167, 169-175, 177-183, and 223-224, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

VIII

Please insert the following new claims:

--58. A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence and one or more amplifiable markers, wherein said vector construct does not comprise a homologous targeting sequence.

59. A vector construct comprising a transcriptional regulatory sequence, an amplifiable marker, and a viral origin of replication.

I 60. A vector construct comprising a selectable marker, a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, and an unpaired splice donor site.

61. A vector construct comprising a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, a sequence-specific protease site, and an unpaired spice donor site.

62. A vector comprising:

(a) a transcriptional regulatory sequence operably linked to a translation start codon,

(b) a nucleic acid sequence encoding an amino acid sequence of four or more amino acids, wherein said amino acid sequence alone is insufficient to constitute signal peptide activity, but is sufficient to constitute signal

(c) over-expressing said endogenous gene in said cell.

78. The method of claim 77, wherein said over-expression is accomplished *in vitro*.

79. The method of claim 77, wherein said over-expression is accomplished *in vivo*.

80. The method of claim 77, further comprising isolating said expression product from said cell.

81. A cell library comprising a collection of cells transformed with the construct of any one of claims 58-62, wherein said construct is integrated into the genomes of said cells by non-homologous recombination.

82. A method of obtaining a gene product from a library of cells comprising screening the library of claim 81 for expression of said gene product, selecting from said library a cell that over-expresses said gene product, and obtaining said gene product from said selected cell.

(I) 83. A method for producing an expression product of an endogenous cellular gene comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence operably linked to a secretion signal sequence and an unpaired splice donor sequence into a cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene or portion thereof; and

(e) culturing said cell under conditions favoring the production of the expression product of said endogenous gene or portion thereof by said cell.

84. The method of claim 83, further comprising isolating said expression product.

85. A method for over-expressing an endogenous gene in a cell *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

86. A method for producing an expression product of an endogenous cellular gene *in vivo*, comprising

(a) introducing a vector comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence into a cell;

(b) integrating said vector integrate into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

87. A method for producing an expression product of an endogenous cellular gene, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene;

(e) culturing said cell under conditions in which said vector and said endogenous gene are amplified in said cell; and

106. The method of claim 77, further comprising introducing double strand breaks into the genomic DNA of said cell prior to or simultaneously with integration of said vector.

107. A gene expression product produced by the method of any one of claims 83, 85-87, 89 and 98.

108. The method of any one of claims 83, 85-87, 89 and 98, wherein said vector construct is linear.

109. A method for producing an expression product of an endogenous gene in a cell comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell in reduced serum medium.

110. A method of protein discovery comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) culturing said cell in reduced serum medium under conditions that allow over-expression of an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence, thereby producing cell-conditioned media; and

(d) screening said cell-conditioned media for the presence of the expression product of said gene or portion thereof.

111. The method of claim 110, further comprising concentrating said cell-conditioned media prior to screening in (d).

112. The method of any one of claims 109-111, wherein said method comprises a high-throughput assay.

I 113. A method for producing an expression product of an endogenous cellular gene comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell under conditions favoring the production of the expression product of said endogenous gene by said cell; and

(f) isolating said expression product from a cell mass equivalent to at least 10 liters of cells at 10^4 cells/ml.

114. The method of any of claims 109-111 and 113, wherein said vector further comprises one or more amplifiable markers.

115. The method of any of claims 109-111 and 113, wherein said vector further comprises an unpaired splice donor site.

116. A method for increasing expression of an endogenous gene in a cell *in situ*, the phenotype of said gene being known, without making use of any sequence information of the gene, the method comprising the steps of:

(a) constructing a vector comprising an amplifiable marker, a transcriptional regulatory sequence, and an unpaired splice donor sequence;

(b) delivering copies of the vector to a plurality of cells;

(c) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells;

(b) integrating a vector comprising one or more transposition signals and a transcriptional regulatory sequence operably linked to an exon-unpaired splice donor complex, into said isolated genomic DNA by transposition, thereby producing a vector-genomic DNA complex;

(c) transfecting said vector-genomic DNA complex into a suitable host cell; and

(d) culturing said cell under conditions suitable to result in protein expression from said genomic DNA contained in said vector-genomic DNA complex.

I 197. A method for expressing a gene, comprising:

(a) isolating genomic DNA, containing one or more genes, from one or more eukaryotic cells;

(b) combining said isolated genomic DNA with a vector comprising:

(i) a selectable marker,

(ii) a transcriptional regulatory sequence operably linked to a translational start codon,

(iii) a secretion signal sequence,

(iv) an epitope tag, and

(v) an unpaired splice donor site,

thereby producing a vector-genomic DNA complex;

(c) introducing said vector-genomic DNA complex into a cell;

(d) selecting for cells containing said vector-genomic DNA complex; and

(e) culturing said cell under conditions suitable to result in expression of a gene contained in said vector-genomic DNA complex.

198. The method of claim 195, wherein said host cell is selected for a cell containing said transfected vector-genomic DNA complex prior to, during, or following being cultured under conditions suitable to result in protein expression.

199. The method of claim 196, wherein said vector further comprises a selectable marker, and wherein said host cell is selected for a cell containing said transfected vector-genomic DNA complex prior to being cultured under conditions suitable to result in protein or gene expression.

200. The method of claim 194, wherein said cloning vector is selected from the group consisting of a BAC, a YAC, a PAC, a cosmid, a phage, and a plasmid.

201. The method of claim 191, further comprising isolating said protein.

202. A protein produced by the method of claim 195.

203. A protein produced by the method of any one of claims 197-199.

204. A protein produced by the method of claim 201.

219. The host cell of claim 218, wherein said vector is integrated into the genome of said cell.

220. The host cell of claim 218, wherein said vector further comprises a viral origin of replication and is maintained within said host cell as an episome.

221. The cell of claim 217 or claim 219, wherein said vector further comprises one or more selectable markers.

222. The cell of claim 219, wherein said viral origin of replication is Epstein Barr Virus oriP.

I 223. A method for activating expression from an endogenous gene comprising:

(a) introducing into a chromosome-containing host cell a vector suitable for activating an endogenous gene;

(b) treating said cell with an agent capable of introducing DNA breaks in the chromosome of said host cell prior to or following introduction of said vector; and

(c) integrating said vector into said DNA breaks so as to result in the formation of an operable linkage between said vector and said endogenous gene, whereby said endogenous gene is activated by one or more vector-encoded nucleotide sequences.

X

Please insert the following new claims:

--58. A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence and one or more amplifiable markers, wherein said vector construct does not comprise a homologous targeting sequence.

59. A vector construct comprising a transcriptional regulatory sequence, an amplifiable marker, and a viral origin of replication.

60. A vector construct comprising a selectable marker, a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, and an unpaired splice donor site.

61. A vector construct comprising a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, a sequence-specific protease site, and an unpaired spice donor site.

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(a) a transcriptional regulatory sequence operably linked to a translation start codon,

(b) a nucleic acid sequence encoding an amino acid sequence of four or more amino acids, wherein said amino acid sequence alone is insufficient to constitute signal peptide activity, but is sufficient to constitute signal

peptide activity when said nucleic acid sequence is combined with or is upstream of an exon of an endogenous gene, and

(c) an unpaired splice donor site.

63. The vector construct of any one of claims 60-62, wherein said construct further comprises one or more amplifiable markers.

64. The vector construct of any of claims 58 and 60-62, wherein said transcriptional regulatory sequence is a promoter.

65. The vector construct of claim 64, wherein said promoter is a viral promoter.

66. The vector construct of claim 65, wherein said viral promoter is a cytomegalovirus immediate early gene promoter.

67. The vector construct of claim 65, wherein said promoter is a non-viral promoter.

68. The vector construct of claim 65, wherein said promoter is an inducible promoter.

69. A cell containing the vector construct of any one of claims 58-62.

70. A cell containing the vector construct of claim 63.

106. The method of claim 77, further comprising introducing double strand breaks into the genomic DNA of said cell prior to or simultaneously with integration of said vector.

107. A gene expression product produced by the method of any one of claims 83, 85-87, 89 and 98.

108. The method of any one of claims 83, 85-87, 89 and 98, wherein said vector construct is linear.

I 109. A method for producing an expression product of an endogenous gene in a cell comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell in reduced serum medium.

I 110. A method of protein discovery comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) culturing said cell in reduced serum medium under conditions that allow over-expression of an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence, thereby producing cell-conditioned media; and

(d) screening said cell-conditioned media for the presence of the expression product of said gene or portion thereof.

111. The method of claim 110, further comprising concentrating said cell-conditioned media prior to screening in (d).

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(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell under conditions favoring the production of the expression product of said endogenous gene by said cell; and

(f) isolating said expression product from a cell mass equivalent to at least 10 liters of cells at 10^4 cells/ml.

114. The method of any of claims 109-111 and 113, wherein said vector further comprises one or more amplifiable markers.

115. The method of any of claims 109-111 and 113, wherein said vector further comprises an unpaired splice donor site.

116. A method for increasing expression of an endogenous gene in a cell *in situ*, the phenotype of said gene being known, without making use of any sequence information of the gene, the method comprising the steps of:

(a) constructing a vector comprising an amplifiable marker, a transcriptional regulatory sequence, and an unpaired splice donor sequence;

(b) delivering copies of the vector to a plurality of cells;

(c) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells;

219. The host cell of claim 218, wherein said vector is integrated into the genome of said cell.

220. The host cell of claim 218, wherein said vector further comprises a viral origin of replication and is maintained within said host cell as an episome.

221. The cell of claim 217 or claim 219, wherein said vector further comprises one or more selectable markers.

222. The cell of claim 219, wherein said viral origin of replication is Epstein Barr Virus oriP.

I 223. A method for activating expression from an endogenous gene comprising:

(a) introducing into a chromosome-containing host cell a vector suitable for activating an endogenous gene;

(b) treating said cell with an agent capable of introducing DNA breaks in the chromosome of said host cell prior to or following introduction of said vector; and

(c) integrating said vector into said DNA breaks so as to result in the formation of an operable linkage between said vector and said endogenous gene, whereby said endogenous gene is activated by one or more vector-encoded nucleotide sequences.